

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>G01N 33/567, C12P 21/06, C12N 5/00, A01N 37/18, C07K 16/00, C07H 21/04</b>		<b>A1</b>	(11) International Publication Number: <b>WO 96/12964</b>
			(43) International Publication Date: <b>2 May 1996 (02.05.96)</b>
(21) International Application Number: <b>PCT/US95/13256</b>			(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).
(22) International Filing Date: <b>10 October 1995 (10.10.95)</b>			
(30) Priority Data: <b>08/326,117 19 October 1994 (19.10.94) US</b>			
(71) Applicant: UNIVERSITY OF WYOMING [US/US]; Albany County, Laramie, WY (US).			Published <i>With international search report.</i>
(72) Inventors: BULLA, Lee, A.; 36645 N. Highway 287, Virginia Dale, CO 80536 (US). JI, Tae, H.; 1067 Granito Drive, Laramie, WY 82070 (US).			
(74) Agents: MURASHIGE, Kate, H. et al.; Morrison & Foerster, 2000 Pennsylvania Avenue, N.W., Washington, DC 20006 (US).			

(54) Title: RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

## (57) Abstract

The cDNA that encodes a glycoprotein receptor from the tobacco hornworm which binds a *Bacillus thuringiensis* toxin has been obtained and sequenced. The availability of this cDNA permits the retrieval of DNAs encoding homologous receptors in other inserts and organisms as well as the design of assays for the cytotoxicity and binding affinity of potential pesticides and the development of methods to manipulate natural and/or introduced homologous receptors and, thus, to destroy target cells, tissues and/or organisms.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

### Acknowledgement of Government Support

Work resulting in the present invention was supported in part by Research Agreement 58-319R-3-011 from the Office of International Cooperation and Development, U.S.D.A. and by Cooperative Agreement 58-5410-1-135 from the Arthropod-Borne Animal Disease Laboratory, Agricultural Research Service, U.S.D.A. and by Grant HD-18702 from the National Institutes of Health. The U.S. government has certain rights in this invention.

### Technical Field

The invention relates to receptors that bind toxins from *Bacillus thuringiensis* and thus to pesticides and pest resistance. More particularly, the invention concerns recombinantly produced receptors that bind BT toxin and to their use in assays for improved pesticides, as well as in mediation of cell and tissue destruction, dissociation, dispersion, cell-to-cell association, and changes in morphology.

### Background Art

It has long been recognized that the bacterium *Bacillus thuringiensis* (BT) produces bacteriocidal proteins that are toxic to a limited range of insects, mostly in the orders Lepidoptera, Coleoptera and Diptera. Advantage has been taken of these toxins in controlling pests, mostly by applying bacteria to plants or transforming plants themselves so that they generate the toxins by virtue of their transgenic character. The toxins themselves are glycoprotein products of the *cry* gene as described by Höfte, H. *et al.* Microbiol Rev (1989) 53:242. It has been established that the toxins function in the brush border of the insect midgut epithelial cells as described by Gill, S.S. *et al.* Annu Rev Entomol (1992) 37:615. Specific binding of BT toxins to midgut brush border membrane vesicles has been reported by Hofmann, C. *et al.* Proc Natl Acad Sci USA (1988) 85:7844; Van Rie, J. *et al.* Eur J Biochem (1989) 186:239; and Van Rie, J. *et al.* Appl Environ Microbiol (1990) 56:1378.

Presumably, the toxins generated by BT exert their effects by some kind of interaction with receptors in the midgut. The purification of a particular receptor from *Manduca sexta* was reported by the present inventors in an article by Vadlamudi, R.K. *et al.* J Biol Chem (1993) 268:12334. In this report, the receptor protein was isolated by immunoprecipitating toxin-binding protein complexes with toxin-specific antisera and separating the complexes by

- 2 -

SDS-PAGE followed by electroelution. However, to date, there has been no structural information concerning any insect receptor which binds BT toxin, nor have, to applicants' knowledge, any genes encoding these receptors been recovered.

#### Disclosure of the Invention

The invention provides recombinant materials for the production of BT toxin-binding receptors as well as methods to employ these materials to generate receptors for use in screening assays for candidate pesticides. Since the native cDNA sequence encoding this receptor, designated BT-R<sub>1</sub>, has been retrieved from the tobacco hornworm, encoding DNA for receptors in other species of insects, as well as in other organisms, which have homology to hornworm receptor can be obtained.

Thus, in one aspect, the invention is directed to a polynucleotide in purified and isolated form which comprises a nucleotide sequence encoding a receptor that binds a BT toxin and other ligands and which has the requisite homology to the BT-R<sub>1</sub> protein.

In other aspects, the invention is directed to expression systems for nucleotide sequences encoding the receptor, to methods of producing the receptor recombinantly, to the receptor as thus produced, to antibodies specifically immunoreactive with the receptor, to assay methods useful for screening candidate pesticides, to antisense polynucleotides corresponding to the coding sequence, to methods of targeting tissues and/or cells using the binding characteristics of the receptor, and to methods of manipulating tissues and/or cells using the function of the receptor.

#### Brief Description of the Drawings

Figure 1 shows the nucleotide sequence and deduced amino acid sequence of cDNA encoding the BT-R<sub>1</sub> protein from *M. sexta*.

Figure 2 shows a comparison of amino acid sequences of cadherin motifs (BTRcad-1 to 11) in BT-R<sub>1</sub> to those of other cadherins.

Modes of Carrying Out the Invention

The invention provides, for the first time, sequence information concerning receptors that bind BT toxins in insect midguts.

The BT-R<sub>1</sub> cDNA clone retrieved as described in the examples below encodes a protein having an identical amino acid composition with that described for the native receptor. Furthermore, toxin-binding specificity and immunoreactivity are similar. The native 210 kD BT-R<sub>1</sub> specifically recognizes *cryIA(b)* toxin of *BT-berliner*; a *K<sub>d</sub>* value of 708 pM was obtained for the native protein.

The *cryIA(b)* toxin selectively kills *M. sexta* larvae with an LC<sub>50</sub> of 7.5 ng/cm<sup>2</sup> of diet surface. BT-R<sub>1</sub> binds the toxin under both reducing and nonreducing conditions and protease treatment of intestinal BBMV vesicles prepared from *M. sexta* showed that a 50 kD fragment of the 210 kD receptor is sufficient for toxin binding. The 50 kD toxin-binding domain is extracellular since the intestinal BBMV vesicles are oriented predominantly right side out as reported by Haase, W.H. *et al.* *Biochem J* (1978) 172:57. This is consistent with the characteristics of the deduced amino acid sequence of the cDNA clone described below, as well as with the binding of toxin to the surface of intact BT-R<sub>1</sub> transfected human embryonic 293 cells as described in Example 3.

Whereas a particular cDNA clone from the tobacco hornworm has been described as illustration, the availability of this sequence information permits retrieval of corresponding receptors responsive to BT and related toxins from other species. This is conveniently accomplished by using the cDNA obtained in the present invention as a probe for screening cDNA or genomic libraries under conditions of stringency which eliminate false positives and retrieve substantially only corresponding receptors with coding sequences that are homologous to the coding sequence for the receptor of the present invention. Thus, the BT-R<sub>1</sub> protein itself and receptor proteins encoded by a nucleotide sequence homologous to the native nucleotide sequence encoding BT-R<sub>1</sub> are provided by the invention. Alternatively, PCR-mediated cloning can be used; however, this method does not take advantage of the detailed and complete information that resides in the availability of the nucleotide sequence encoding the full-length receptor from *M. sexta*. Also, PCR-mediated cloning introduces

- 4 -

errors in natural DNA sequences. Thus, by using the full-length cDNA as a probe under conditions of appropriate stringency, only nucleotide sequences encoding the corresponding receptors will be obtained. The standard hybridization conditions include hybridization with nonspecific DNA such as salmon DNA at 500C and washing at 450C. To obtain corresponding receptors having the lowest detectable homology with the receptor from *M. sexta*, the cDNA probe is hybridized under conditions of low standard stringency (30-370C and 4-6X SSC. More closely related corresponding receptors are obtained by hybridizing the cDNA probe under moderate standard stringency conditions (40-500C in 1X SSC).

The distribution of receptors of appropriate homology in the animal kingdom is believed to be fairly wide. Indeed, it is thought that higher organisms such as mammals, including primates, contain corresponding receptors which are homologous to BT-R, but respond to modified forms of BT toxins. In addition, other parasites such as nematodes, both those that afflict plants and those that afflict animals, will contain corresponding receptors.

Although one of the advantages of the use of BT toxins as insecticides is its specificity for certain orders of insects, this specificity is believed to result from the particular structure of the BT toxin rather than the unavailability of a corresponding mechanism in other insect orders. Thus, modified forms of BT toxin would be effective with respect to insects which contain homologous but slightly different forms of the receptor from that of the BT-R, protein illustrated below.

As used herein, "A receptor that specifically binds a BT toxin" refers to a receptor which is homologous to the BT-R, protein illustrated herein and which binds to either BT toxins themselves or to BT toxins that are sufficiently modified so as to bind these receptors which provide the required homology to BT-R.

The criteria for inclusion of a receptor in the present invention are the requirements that 1) it behave as a receptor -- i.e., be capable of being displayed at the cell membrane; 2) it be sufficiently homologous to the BT-R, receptor described herein that a nucleotide sequence encoding the protein hybridizes under the stringency conditions described above to the nucleotide sequence encoding BT-R, as shown in Figure 1; and 3) when displayed on the surface of a cell, it is capable of binding a BT toxin or a modified form of BT toxin that exerts

- 5 -

a cytotoxic effect either on the cell in which the receptor resides or in a tissue with which the cell is associated.

The structural characteristics of the "modified BT toxin" are defined by the functional property set forth above, but it may be convenient to design modified forms of BT toxin by conservative amino acid substitutions or other known protein-manipulating techniques applied to naturally occurring BT toxins.

The presence of similar receptors in noninsect organisms as well as other insects besides those harboring BT-R<sub>1</sub> is supported by the sequence similarity of the BT-R<sub>1</sub> protein to that of the various members of the cadherin superfamily of proteins, which are membrane glycoproteins believed to mediate calcium-dependent cell aggregation and sorting. See, for example, Takeichi, M. Science (1991) 251:1451; and Takeichi, M. N Rev Biochem (1990) 59:237.

Included in this superfamily are desmogliens, desmocollins, the *Drosophila* fat tumor suppressor, human intestinal peptide transport protein and T-cadherin. All of these proteins share common extracellular motifs although their cytoplasmic domains differ. Goodwin, L. *et al.* Biochem Biophys Res Commun (1990) 173:1224; Holton, J.L. *et al.* J Cell Sci (1990) 97:239; Bestal, D.J. J Cell Biol (1992) 119:451; Mahoney, P.A. *et al.* Cell (1991) 853; Dantzig, A.H. *et al.* Science (1994) 264:430; and Sano, K. *et al.* EMBO J (1993) 12:2249. Inclusion of BT-R<sub>1</sub> in the cadherin superfamily is further supported by the report that EDTA decreases the binding of *cryIA(b)* toxin of BT to the 210 kD receptor of *M. sexta* (Martinez-Ramirez, A.C. *et al.* Biochm Biophys Res Commun (1994) 201:782).

It is noted below that the amino acid sequence of BT-R<sub>1</sub> reveals that a calcium-binding motif is present. This is consistent with the possibility that cells having receptors to bind toxin may themselves survive although they render the tissues in which they are included permeable to solutes and thus effect disintegration of the tissue. Such a mechanism is proposed for the death of insects that ingest the toxin via the epithelial cells in their midgut by Knowles, B.H. *et al.* Biochim Biophys Acta (1987) 924:509. Such a mechanism is also supported in part by the results set forth in Example 4 hereinbelow which indicate that the effect of the toxin on embryonic 293 cells modified to express the receptor at their surface is reversible.

- 6 -

Thus, in summary, the invention provides a family of receptors that is able to mediate the negative effects exerted by BT toxin or its modified forms on the cells expressing the receptor, by damaging the cells themselves and/or the tissue or organ of which the cells form a part. The receptor may be expressed natively at the surface of the target cells or the target cells may be modified to contain an expression system which will effect the display of receptor at the surface. The availability of this family of receptors and recombinant methods for its production and for the production of cells displaying it at their surfaces provides a number of opportunities to conduct screening assays for improved toxins, particularly insecticidal toxins, for generation of antibodies that can be useful as alternatives to chemotherapeutic agents for the destruction and/or dissociation of unwanted cells or tissues, and for the design of improved toxins and pharmaceuticals.

Screening Assays

The availability of the recombinant family of receptors of the present invention permits design of straightforward screening assays for toxins which will interact successfully with these receptors resulting in measurable effects on the cells in which the receptors reside. Briefly, suitable host cells, such as COS cells for transient expression, CHO cells for stable expression, and a variety of other mammalian and insect host cells can be modified to contain expression vectors appropriate to the hosts for the production of the receptors of the invention displayed on the surfaces of the cells. Since the receptors are natively membrane proteins, no particular design of the expression system is required in order to effect their disposition at the cell surface. Expression vectors suitable for any desired host are generally known in the art. For example, for mammalian expression, suitable control sequences include the SV40 and adenovirus promoters as constitutive promoters, the metallothionein inducible promoter, suitable enhancers, if desired, and termination signals and the like. For insect cells, the baculovirus system is preferred. For other eucaryotic cells such as yeast, the glycolytic enzyme promoters and various amino acid synthesis promoters are commonly employed. Prokaryotic cells such as *E. coli* also may be adapted for expression of the receptor in the assay of the invention, for instance by using a reporter gene under the control of cyclic AMP and operably linked to the receptor via protein G such that toxin binding will interrupt adenyl cyclase activity and thereby produce a detectable change in reporter gene activity. The assay system in a prokaryotic host may require further modification to compensate for lack of glycosylation which is known to occur in insect cells where the BT-R<sub>1</sub> protein is naturally expressed.

The cells are modified by transfection, retroviral infection, electroporation or other known means, to contain the desired expression system and then cultured under conditions wherein the receptor protein is produced and displayed. If desired, the cells are then recovered from the culture for use in the assay, or the culture itself can be used *per se*.

In the assays, the modified cells are contacted with the candidate toxin and the effect on metabolism or morphology is noted in the presence and absence of the candidate. The effect may be cytotoxic -- i.e., the cells may themselves exhibit one of the indices of cell death,

such as reduced thymidine uptake, slower increase in optical density of the culture, reduced exclusion of vital dyes (e.g., trypan blue), increased release of viability markers such as chromium and rubidium, and the like. The differential response between the toxin-treated cells and the cells absent the toxin is then noted. The strength of the toxin can be assessed by noting the strength of the response.

These assays may be conducted directly as described above or competitively with known toxins. For example, one approach might be to measure the diminution in binding of labeled BT cry toxin in the presence and absence of the toxin candidate.

In addition to simply screening candidates, the screen can be used to devise improved forms of toxins which are more specific or less specific to particular classes of insects as desired. The ability to determine binding affinity ( $K_a$  and  $K_d$ ), dissociation and association rates, and cytotoxic effects of a candidate allows quick, accurate and reproducible screening techniques for a large number of toxins and other ligands under identical conditions which was not possible heretofore. Such information will facilitate the selection of the most effective toxins and ligands for any given receptor obtained from any desired host cell.

Competition assays may also employ antibodies that are specifically immunoreactive with the receptor. Such antibodies can be prepared in the conventional manner by administering the purified receptor to a vertebrate animal, monitoring antibody titers and recovering the antisera or the antibody-producing cells for immortalization, to obtain immortalized cells capable of secreting antibodies of the appropriate specificity. Techniques for obtaining immortalized B cells and for screening them for secretion of the desired antibody are now conventional in the art. The resulting monoclonal antibodies may be more effective than the polyclonal antisera as competition reagents; furthermore, the availability of the immortalized cell line secreting the desired antibody assures uniformity of production of the same reagent over time. The information and the structural characteristics of toxins and ligands tested will permit a rational approach to designing more efficient toxins and ligands. Additionally, such assays will lead to a better understanding of the function and the structure/function relationship of both toxin/ligand and BT-R<sub>1</sub> analogs. In turn, this will allow the development of highly effective toxins/ligands. Ligands include natural and modified

- 9 -

toxins, antibodies (anti-receptor and antiidiotypic antibodies which mimic a portion of a toxin that binds to a receptor, and whatever small molecules bind the receptors.

### Therapeutic Strategies

Advantage may be taken of the ability of receptors to mediate the destruction, dissociation or association of cells, tissues or organs by utilizing the screening assay as a method to identify successful therapeutics in the treatment of, for example, malignancies, metastases and infectious microorganisms which naturally express receptors corresponding to BT-R<sub>1</sub>. The presence of receptors corresponding to the BT-R<sub>1</sub> receptor illustrated herein and members of the family of receptors included in the invention in the undesired cells may be exploited by first assessing the interaction of a proposed therapeutic with the receptors on these cells in culture and then identifying agents which successfully interact with the receptors as useful candidate reagents. Antibodies reactive with these receptors comprise a class of promising therapeutic candidates.

In some applications target cells, tissues, organs, and microorganisms which do not express an effective receptor corresponding to the BT-R<sub>1</sub> receptor may be transformed or transfected to express an effective corresponding receptor. These targets then will be killed or manipulated with toxin or other ligands. For instance, yeast cells to be used for toxin assays for a particular insect may be transformed with a genetic construct for expression of the receptor from that insect which corresponds to the BT-R<sub>1</sub> receptor.

In another aspect of the invention the receptors corresponding to BT-R<sub>1</sub> in certain target cells may be manipulated by modified toxin or other ligands to prevent the normal response to toxin (dissociation, damage and death of membranes, cell, tissues and organisms). For instance, a ligand which binds to a corresponding receptor in such a way that normal receptor function is inhibited would thereby prevent the receptor from initiating the usual destructive effects in the presence of a normal ligand such as a toxin. In other words, the invention enables development of competitive inhibitors of a toxin or other ligand which normally initiates destructive or other effects via a receptor corresponding to BT-R<sub>1</sub>.

The following examples are intended to illustrate but not to limit the invention.

- 10 -

Example 1

Purification and Sequence Determination of BT-R<sub>1</sub> Protein

Midguts of *M. sexta* were extracted and the BT-R<sub>1</sub> protein purified according to the method of Vadlamudi, R.K. *et al.* J Biol Chem (1993) 268:1233, referenced above and incorporated herein by reference. The electroeluted band was confirmed to contain BT-R<sub>1</sub> protein by binding to <sup>125</sup>I-cryIA(b) toxin. In gel electrophoresis, the protein bound to toxin had an apparent weight of approximately 210 kD under reducing and nonreducing conditions.

The purified electroeluted BT-R<sub>1</sub> was subjected to cyanogen bromide digestion and the cyanogen bromide fragments separated on a 17% high-resolution tricine SDS-polyacrylamide gel as described by Schagger, H. *et al.* Anal Biochem (1987) 166:368. The separated fragments were transferred to Problott membranes (Applied Biosystems) and five bands were extracted and subjected to microsequencing using standard instrumentation. The amino acid sequences obtained were:

1. (Met)-Leu-Asp-Tyr-Glu-Val-Pro-Glu-Phe-Gln-Ser-Ile-Thr-Ile-Arg-Val-Val-Ala-Thr-Asp-Asn-Asn-Asp-Thr-Arg-His-Val-Gly-Val-Ala;
2. (Met)-X-Glu-Thr-Tyr-Glu-Leu-Ile-Ile-His-Pro-Phe-Asn-Tyr-Tyr-Ala;
3. (Met)-X-X-His-Gln-Leu-Pro-Leu-Ala-Gln-Asp-Ile-Lys-Asn-His;
4. (Met)-Phe/Pro-Asn/Ile-Val-Arg/Tyr-Val-Asp-Ile/Gly;
5. (Met)-Asn-Phe-Phe/His-Ser-Val-Asn-Arg/Asp-Glu.

- 11 -

Example 2  
Recovery of cDNA

An *M. sexta* cDNA library was constructed from midgut tissue in Sg10 using the Superscript Choice System according to the manufacturer's instructions (Life Technologies, Inc.). Degenerate oligonucleotide probes were constructed based on the peptide sequences determined in Example 1 using the methods and approach described in Zhang, S. *et al.* Gene (1991) 105:61. Synthetic oligonucleotides corresponding to peptides 1-3 of Example 1 were labeled with  $K^{32}P$  using polynucleotide kinase and used as probes as described in the standard cloning manual of Maniatis, T. *et al.* Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2nd ed. 1989). A clone hybridizing to all three probes identified from 40 positive clones as hybridizing to all three of the probes was plaque-purified from a screen of  $4 \times 10^5$  recombinants and subcloned into pBluescript (Stratagene). It contained an insert of 5571 bp.

Double-stranded cDNA in pBluescript was sequenced in both directions by the dideoxy termination method with Sequanase (USB) according to the manufacturer's instructions. The sequencing showed an open reading frame of 4584 base pairs or 1528 amino acids along with a polyadenylation signal at position 5561. The sequence obtained and the deduced amino acid sequence is shown in Figure 1.

Thus, the deduced protein has a molecular mass of 172 kD and a pI of approximately 4.5. The amino acid sequences of the cyanogen bromide fragments of native receptor match perfectly within the deduced amino acid sequence. The open reading frame begins with an ATG that is flanked by the consensus translation initiation sequence GAGATGG for eucaryotic mRNAs as described by Kozak, M. Nucleic Acids Res (1987) 15:8125.

As shown in Figure 1, the deduced amino acid sequence includes a putative signal, shown underlined, preceding the mature N-terminus Asn-Glu-Arg-etc. Eleven repeats (cad1-cad11) are shown in the extracellular region upstream of the membrane domain, shown with the heavy underline, at positions 1406-1427. The end of the 11th repeat is shown with an arrowhead. The positions of the five CNBR fragments are also shown under the complete sequence.

- 12 -

Figure 2 compares the BT-R<sub>1</sub> sequence obtained herein with other members of the cadherin family. Like known cadherins, the external domain of BT-R<sub>1</sub> is highly repetitive and contains 11 repeats (cad1-cad11; see Figure 2 A). The other cadherins compared in Figure 2 B are mouse P cadherin (mP EC1); *Drosophila fat* EC18 (fat EC18) and protocadherin (PC42 EC2), and human intestinal transporter (HPT-1-EC-1). The eleven repeats of the cadherin motif in BT-R<sub>1</sub> (cad1-cad11) are individually aligned with a single motif sequence from each of the other members of the cadherin family. Conserved residues are boxed. The greatest similarity of BT-R<sub>1</sub> to the cadherins is with the extracellular repeats of the cadherin motif of mouse P-cadherin, *Drosophila fat* tumor suppressor and the protocadherins, although homologies are not high (20-40 homology and 30-60 percent similarity). The conserved repeats of BT-R<sub>1</sub> included AXDXD, DXE, DXNDXXP, one glutamic acid residue and two glycine residues (Figure 2 B). Motifs A/VXDXD, DXNDN are the consensus sequences for calcium binding and two such regions are present in a typical cadherin repeat. In all repeats of BT-R<sub>1</sub>, the sequence DXNDN is preceded by 8 to 14 hydrophobic amino acids. Similar hydrophobic sequences also have been observed in the cadherins. The length of the hydrophobic stretches suggests that these areas are not transmembrane regions but that they represent J-sheet structures commonly present in cadherin-like repeats. BT-R<sub>1</sub> contains a putative cytoplasmic domain of 101 amino acids, smaller than vertebrate cadherin cytoplasmic domains (160 amino acids), and shows no homology to any of the cadherin cytoplasmic domains or to cytoplasmic domains of other proteins to which it has been compared in a current sequence data base.

To confirm that the sequenced clone encoded full-length BT-R<sub>1</sub> protein, total mRNA was prepared from midguts of *M. sexta* subjected to Northern blot by hybridization with the antisense 4.8 kb SacI fragment of the BT-R<sub>1</sub> cDNA clone. The Northern blot analysis was conducted by hybridizing to the antisense probe at 420C and 50% formamide, 5 X Denhardt's Reagent, 5 X SSCP and 50 Tg/ml salmon sperm DNA. The filter was then washed two times with 1 X SSC + 0.1% SDS and two times with 0.15 X SSC + 0.1% SDS at 420C. Each wash was roughly 20 minutes. The filter was then exposed to X-ray film for 24 hours. The 4.8 kb probe hybridized to a single 5.6 kb band.

- 13 -

The BT-R<sub>1</sub> clone was translated using rabbit reticulolysate and the resulting translated products were immunoprecipitated with antisera raised against native protein encoded by BT-R<sub>1</sub>. For the *in vitro* translation, pBluescript plasmid containing BT-R<sub>1</sub> cDNA was linearized and transcribed with T<sub>3</sub> polymerase (Pharmacia). The translation was conducted according to manufacturer's instructions with nuclease-treated rabbit reticulolysate (Life Technologies, Inc.). After one hour of incubation at 300C, the reaction mixture was combined with an equal volume of SDS buffer or lysed with 50 mM Tris buffer containing 1% NP40 and 250 mM NaCl (pH 8.0) for immunoprecipitation. Preimmune serum was used as a control. Translation and immunoprecipitation products were electrophoresed on a 7.5% SDS-polyacrylamide gel fixed, treated with Enhance (Dupont NEN), dried and exposed to X-ray film for 12 hours.

Two protein bands of approximately 172 kD and 150 kD as determined by SDS-PAGE were obtained; it is postulated that the 150 kD translation product was due to initiation of translation from an internal methionine at amino acid 242. This is consistent with the observations of Kozak, M. Mol Cell Biol (1989) 9:5073.

Thus, both results confirm that a full-length clone was obtained.

### Example 3

#### Recombinant Production and Characteristics of the BT-R<sub>1</sub> Protein

The BT-R<sub>1</sub> cDNA clone was subcloned into the mammalian expression vector pcDNA3 (Invitrogen) and the construct transfected into COS-7 cells. Membranes isolated from the COS-7 transfectants were solubilized, electrophoresed and ligand blotted with <sup>125</sup>I-*cryIA(b)* toxin. The cells were harvested 60 hours after transfection, washed with phosphate-buffered saline and lysed by freezing in liquid nitrogen. Cell membranes were prepared by differential centrifugation as described by Elshourbagy, N.A. *et al.* J Biol Chem (1993) 266:3873. Control cells were COS-7 cells transfected with pcDNA3.

The cell membranes (10 Tg) were separated on 7.5% SDS-PAGE blotted to a nylon membrane and blocked with Tris-buffered saline containing 5% nonfat dry milk powder, 5% glycerol and 1% Tween-20. The nylon membrane was then incubated with <sup>125</sup>I-*cryIA(b)* toxin

- 14 -

(2 X 10<sup>5</sup> cpm/ml) for two hours with blocking buffer, dried and exposed to X-ray film at -700C. The labeled toxin bound to a 210 ± 5 kD protein; the 210 kD band was observed only in lanes containing membranes prepared from either *M. sexta* or COS-7 cells transfected with the BT-R<sub>1</sub> cDNA construct containing 4810 bp of cDNA comprising the open reading frame.

The discrepancy between the 210 kD protein expressed and the calculated 172 kD molecular weight is due to glycosylation of the protein; *in vitro* translation of the cDNA clone, as described above, which does not result in glycosylation, does produce the 172 kD protein. To verify this, the COS-7 produced protein was subjected to digestion with N-glycosidase-F by first denaturing the purified protein by boiling in 1% SDS for 5 minutes followed by addition of NP-40 to a final concentration of 1% in the presence of 0.1% SDS, and then incubating the denatured protein in sodium phosphate buffer, pH 8.5 at 370C with N-glycosidase-F for 10 hours. Controls were incubated under the same conditions without enzyme. Digestion products were separated on a 7.5% SDS-PAGE and stained with Coomassie brilliant blue. This glycosidase treatment reduced the molecular weight of BT-R<sub>1</sub> protein from 210 to 190 kD; this indicates N-glycosylation at some of the 16 consensus N-glycosylation sites in the protein. Treatment of BT-R<sub>1</sub> with O-glycosidase and neuraminidase did not alter the mobility of the protein.

In addition, embryonic 293 cells were transfected with the BT-R<sub>1</sub> cDNA clone in pcDNA3 and incubated with the labeled toxin (0.32 nM) in the presence of increasing concentrations (0 to 10<sup>-6</sup> M) of unlabeled toxin. Nonspecific binding was measured as bound radioactivity in the presence of 1 TM unlabeled toxin. A value for the dissociation constant (K<sub>d</sub>) of 1015 pM was determined by Scatchard analysis; this is approximately the same value that was obtained for the natural receptor as described by Vadlamudi, R.K. *et al. J Biol Chem* (1993) (*supra*).

- 15 -

Example 4

Physiological Effect of BT Toxin  
on Modified Embryonic 293 Cells

Both unmodified embryonic 293 cells, and 293 cells which have been modified to produce the BT-R<sub>1</sub> receptor as described in Example 3, when cultured *in vitro* form adherent star-shaped clusters. When BT toxin (200 nM) is added to serum-free medium, the clusters round up and release from the plastic surfaces of the culture dish. This effect is also observed under known conditions of cytotoxicity for 293 cells. The foregoing effect is observed only when the cells are cultured in serum-free medium since the toxin binds to serum and would thus be ineffective under conditions where serum is present.

However, in the presence of anti-receptor antisera, this effect of BT toxin is blocked. Also, when serum is added back to a culture of modified E293 cells which has been treated in serum-free conditions with the toxin, the cells revert to their normal star-shaped adherent cluster shapes. This indicates that the effect of the toxin is reversible.

- 16 -

Claims

1. A polynucleotide in purified and isolated form which comprises a nucleotide sequence which encodes a receptor that specifically binds a *Bacillus thuringiensis* (BT) toxin wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1.
2. The polynucleotide of claim 1 wherein said toxin is the *cryLA(b)* toxin of *B. thuringiensis* subsp. *berliner*, or wherein said toxin is a modified form of BT toxin; or wherein said receptor is the BT-R, receptor of the tobacco hornworm *Manduca sexta*.
3. A recombinant expression system for expression of a nucleotide sequence encoding a receptor which specifically binds a BT toxin, wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1,  
which expression system comprises said encoding nucleotide sequence operably linked to control sequences operable in host cells.
4. Recombinant host cells modified to contain the expression system of claim 3.
5. A method to produce a receptor which binds a BT toxin which method comprises culturing the cells of claim 4 under conditions wherein said receptor is produced; and optionally recovering said receptor from the culture.
6. The method of claim 5 wherein said receptor is disposed at the surface of said cells, and wherein the cells are optionally recovered from the culture for use in an assay.

- 17 -

7. Cells expressing BT toxin receptor disposed at their surface prepared by the method of claim 6.

8. A method to assess the binding affinity or cytotoxicity of a candidate pesticide for an insect receptor that binds BT toxin which method comprises contacting said candidate with the cells of claim 7 under conditions which permit binding to occur; and  
detecting the presence, absence or amount of said candidate bound to said cells, or  
assessing the cytotoxicity of said candidate with respect to said cells.

9. A receptor which binds BT toxin produced by the method of claim 5.

10. A purified and isolated polynucleotide which comprises a nucleotide sequence complementary to a nucleotide sequence encoding a receptor which binds to BT toxin wherein said receptor has the amino acid sequence of the receptor shown in Figure 1, or wherein said receptor is encoded by a nucleotide sequence that hybridizes under conditions of standard stringency to the nucleotide sequence shown in Figure 1.

11. A composition which comprises antibodies specifically immunoreactive with a receptor that specifically binds a BT toxin, said receptor encoded by the polynucleotide of claim 1.

12. A method to modify target cells so as to render them susceptible to interaction with a BT toxin which method comprises modifying said cells to contain the expression system of claim 3; and

culturing the cells under conditions wherein said receptor is disposed at the surface of the cells.

13. Cells modified by the method of claim 12.

- 18 -

14. A method to exert a cytotoxic effect on target cells or on a tissue with which said target cells are associated; therein said target cells include, at their surface, a receptor that specifically binds BT toxin, which method comprises contacting said cells with the antibody composition of claim 11, or with an amount of BT toxin effective to interact with said receptor.

100. GACCAATCGGAACTGGGGAAATTGGAAATTGGGGTTCCCTTCTGGTTAGTTAGTTACCTTGTAAATAAGTACTTTAGTTACAAATTGGAAATAATTGGCAGC  
AAAACCATCTGCAAGCAACAAATCATCTGAGCTGCGAAATCATCTGAGCTCTCAGGAGCAAAAGCATCTCAGGAGCAGAAACCCCCAAATAATGTGAG ATG  
M>

200. GCA GTC GAC GTC CGA ATC GCT GCC TTC CTC CTG CTG GTC TTT ATA GCG CCT GCA GTT TTA GCT CAA GAG AGA TGT GGG  
A V D Y R A F A P L V F A P A V L A P Q E R C G>

300. TAT ATG ACC GCC ATC CCA AGG CTA CCA CGA CCG GAT AAT TTG CCA GTC CTA AAT TTT GAA GGC CAG ACA TGG AGT  
Y M T A P R L P D N F V L N F T W S>

400. CAG AGG CCC CTG CTC CCC GCC CCG GAG CGG GAT GAC CTG TGC ATG CAC GCC TAC ACA GCC AAC CTC  
Q R P L P A P E R D D C M D Y H V I T A N L>

500. GGC ACG CAG GTC ATC TAC ATG GAT GAA GAC AIA GAA GAG ATC ACC ATC GCC ATC CTT AAT TAT AAC GGA CCA  
G T Q V D E E D E I T A P V R R Y L N Y N G P>

600. TCA ACT CCG TTC ATT GAA CTG CCA TTT TTA TCC GGT TCG TAC AAT CTG CTG ATG CCG GTC ATC AGG AGA GTT GAC  
S T P F I E L S G S Y N L L M P V I R R V D>

N G S A R Q H A H Y M F Q Q V>

**SUBSTITUTE SHEET (RULE 26)**

FIG. IA

CGC GAC GGC CAG TCG CTC GCA GGC GTC TCT CTC GCT ATC GTC AAC ATA GAT GAC AAC CCC ATC ATA  
 R V D G Q L V A G V S L A V D N A P I >  
 CAA AAC TIC GAG CCT TGC CGG GTT CCT GAA CTG GGC GAG CCA GGG TTG ACA TAC CAA GTA TCG TAC  
 Q N F E P C R V D E L G E P G L T E C T Y Q V S D >  
 700.  
 GCG GAC GGA CGG ATC AGC ACA GAG TTC ATG AGG ATC GAC AGC GTC GAG GAG ACC TAC AAC TIC  
 A D G R F I S T E M F R S D E E >  
 800.  
 ATC GAA CGG ACG AAT ATC CCC AAC CAA TGG ATG TGG CTA AAT ATG AGC ATC GTC GAT AAC TIC  
 I E R R I F N Q W M W M V N T S L >  
 900.  
 GTC ACC AGT CGG CTG CAT ATA TIC AGC GTC GCA GGC CTG GAC TCG CTC CCG AAC ACC CAC ACG GTG ACT ATG ATG  
 V P L H S V A L D S L P N T H V T M M >  
 GTG CAA GTG GCG AAT GTG AAC AGC CGT CGG CCG GAG ATC TIC GTC GCT GTC CAA CAG TTT GAA GAG AAA  
 V Q A N V N S R P R W L E I F A V Q F E E K >  
 1000.  
 TCT TAC CAA AAC TTC ACA GTG AGG GCG ATC GAC GGA GAC ACT GAG ATC AAC TAC AGG CTG ATC  
 S Y Q N F T V D G D T E I N H P Y R L >  
 1100.  
 ACA AAT GAG GAA GAC ACA TIC TIC AGC ATT GAG GCC CCT GGT GGA AAA AGC GGC CTC GTC TCG  
 T N E D E F S G K >  
 1200.  
 2/12

IB  
FIG.

1300\*  
 CCA ATT GAC CGC GAC ACA CTC CGA GAG GTG TTT CCA CTT ACG ATC GTC GCT TAC AAA TAT GAT GAG GAG GCC  
 P D R Q L T F P V A T Y K D E E A>  
 1312  
 TTC TCC ACA TCA ACA AAC GTC GTC ATC ATT GTG ACA AAC GAC CAA ACA CCT GAA CCT ATA CAC AAG GAA  
 F S T N V V D I N Q R P E H K E>

1400.  
 TAT CGA CTG GCA ATC ATG GAG GAG ACG GTG CTT CTA GAG AGC CTC AAC TTC CAT AAA GAA TTC GGA TTT CAT GAT AAG GAT  
 Y R L M E E T P L N F D K E F G F H D K D>

1500.  
 TTA GGT CAA AAC GCT CAG TAC ATG GTG CTT CTA GAG AGC CTC AAC TTC CAT AAA GAA TTC GGA TTT CAT GAT AAG GAT  
 L G Q N A Q D E S V A T Y K D E A F Y D>

1512  
 1600.  
 GCG CCT GAA GTC GGC TAC CAG CGA CAG ACC TTC ATC ATG CCC ACC CTC ATT CAC TCC ATG CTG GAT TAC GAA GTC  
 A P E V G Y Q R Q L N H S M D Y E V A>  
 1612

CCA GAG TTT CAG AGT ATT ATG ATT CGG GTG GCA ACC GAC AAC AAC GAC ACC AGG CAC GTC GGC GTC GGC TIG  
 P E F S T Q S I R V V A T D N N D T R H V G V A L>

1700.  
 GTT CAC ATT CAC CTC ATC ATT TGG AAC GAT GAG CAG CGG ATC TTC GAA CAC GCC GTG CAG ACC GTC ACC TTC GAC  
 V H D L N W D E Q P F V Q A V F D>

1800.  
 GAG ACT GAA GCC GAG GGG TTC TTC GTC GCC AAG GCG GTC GCA CAC GAC AGA GAC ATC GGG GAT GTC GTC GAG CAT  
 E G E G F G V A K D R G D V Y F H>

**FIG. 1C**

FIG.

AAC GCG CCC GTG TGG GAG GGG ACT CTG GAG CAG AAC TTC CGA GTC CGC CAG ATG TCG GCG GGG CTC GTG  
 N A P V W E F D G Y S R A D G P L Y N Q V R Y P F T C A A C G G A C C G A C A T C G A C I D G  
 GTG GGC TCC GTG CGC GCG GAC GAC ATC GAC CCG CTC TAC AAC CAA GTC CGA TAC ACC ATT TTC P R Y C G T G A A  
 V G S V R A D D I D G  
 2500.

GAC ACA GAT AAG GAC CTG ATA ATG ATC GAA CTT CCT CAC GGG TCA AAT TTC CGT GAA CAC AAG CGG CGC ATC GAC  
 D T B K D L I M P H  
 2600.

GCG AAT ACT CCT CCA CGC TIC CAC CTC TAC TAT ACA GTG GCT AGT GAC CGA TGC TCG ACA GAA GAT CCT GCA  
 A N F T P R  
 2700.

GAT TGC CCT GAC CCG ACT TAT TGG GAA ACC GGA AAT ATC ACA ATC CAC ATC ACC GAC ACG AAC AAC AAG  
 D C P D Y W E G N T I H  
 GTC CGG CAG CGG CAA ACC ACT AAG TTC GAT ACC GTC GTG TAT ATT TAC GAG AAC GCA ACC CAC TTA GAC GAG GTG  
 V P Q A E T K F D Y V  
 2800.

GTC ACT CTG ATA GCC AGT GAT CTT GAC AGA GAC GAA ATA TAC ACC ATG GTG AGC TAC GTC ATC ATT TAT GCA GTC  
 V L A S D  
 2900.

AAC CCT CGA CTG ATG AAC TIC TIC TCC GTC AAC CGA GAG ACC CGC CTC GTC GAC TAT GAG ACC CAG GTG  
 N P R M N F S V E  
 3000.

5/12

FIG. 1E

3100. AGT GGC CAG GTG CTG CAC CGT CAT GGT GAT GAA CCA ACG CAC CGT ATC TTC AAC CTC ATC GAC AAC TTC ATG  
 S G E V L D R G D P E F N L D N F M>  
 GGG CAA GCA GAA GGT AAC AGA AAT CAG AAC GAC ACA GAA GTT CTC GTT ATC TTC GAT GTG AAT GAC AAT GCT  
 G E G N R N D Q E V L D N D N A>  
 3200. CCT GAA TTG CCA CCG CCG AGC GAA CTC TCT TGG ACT ATG TCT GAG AAC CTT AAG CAG GGC GTC CGT CTT GAA CAA  
 P E L P P S E L S E N L K Q G V R L E P>  
 3300. CAT ATC TTC GCC CGC GAC CGC GAC CGC GAG CCC GAC ATC AAC TCC AGG GTC GGC TAC GAG ATC CTG AAC CTC AGC  
 H F A P D R D E P D N S R V G E L N S>  
 3400. 6/12  
 ACG GAG CGG GAC ATC GAA GTG CCG GAC CTC TTT GTG ATG ATG CAG ATC GCG AAC GTC V ATC GCG GGA GAG CTG GAG ACC  
 T E R D I E V P E L F V M I Q G A N V T G E L E T>  
 GCC ATG GAC CTC AAG GGA TAT TGG GGG ATG TAC ATG TAT ATG CTC GCA TTC GAC GGC ATT CCG CAA ATG  
 A M D L K G G Y W G I A P H F D H C A F G P G M>  
 3500. TCC ATG AAC GAG ACA TAT GAG CTG ATC AAC TAC TAC GCG CCT GAG CCT GTC GTC GTC CCG AAC  
 S M N E T Y E L P F N Y A P E F V F P T C C G A C C A A C  
 3600.  
 GAT GCC GTC ATG CGA CTT GCG AGG GAA CGA GCT GAA ATC AAT GGA GTT GIA GCG ACA GTG AAC GGA GAG TTC TIG  
 D V R L A R E R A V A N G V A L A G V F F L>

**FIG. IF**

3700. GAG CGG ATA TCG GCG ACT CAT CCG GAC GGA CTC CAC GCG GTC GTC ACC TTC CAA GTG GTC GAT GAG GAA  
 E R S A D P T H L G V F Q D N E G S L G S L R L Q A V P E>  
 TCA CAA CGG TAC TTT CAA GAA GAT GGC AAC CTC GGC TCG TIG AGG TAA CAA CCC GTT CCA GAG  
 S Q R Y F Q V D N E P L G S L R L Q A V P E>  
 3800. GAG ATC AGG GAG TTC CGG ATA ACG ATT CGC GCT ACA GAC CAG GGA ACG GAC CCA GGA CGG CTG TCC ACG GAC ATG  
 E R S F E P R T V P T D Q G E P R F A S E H A V A F D M>  
 3900. ACG TTC AGA GTT GTT GTG CCC ACG CAA GGA GAA CCT AGA TTC GCG TCC TCA GAA CAT GCT GTC GCT TTC AIA  
 T F R V F V P Q G E P R F A S E H A V A F D M>  
 4000. 7/12  
 GAA AAG AGT GCC GGC ATG GAA GAG TCT CAC CAA CCT CCT CTA GCT CAA GAC AAG AAC CAT CTC TGT GAA GAC  
 E K S A G M E H Q D P A Q D I K N H F L C E D>  
 GAC TGT CAC AGC ATT TAC TAT CGT ATT ATC GAC GGC AAC AGC AAC GAA GGT CAT TTC GGC CTG GAT CCT GTT CGC AAC  
 D C H S P K E D G E H F G L D P V R N>  
 4100. AGG TIG TTC CIG AAA GAG CIG ATA AGG GAA CAA AGT GGC CAC ACT CIG CAA GTG GCG GCT AGT AAC TCG  
 R L F K K P E L R E Q S H A V A F P R S N S>  
 4200. CCC GAT GGT GGC ATT CCA CCT CCT GCT ACT GTC ACT GTT ACC GTG AGG GAG GCA GAC CCT CGT CCA  
 P D G G P A S P A T V R E A D P R S P>

**FIG. 1G**

4300\*  
 GTG TTT GTG AGG CAA TTC TAC ACC GCA GGG A<sub>T</sub> A<sub>C</sub> GCA GAC TCC ATC GGC AGA GAG CTG CTC AGA TTA CAT  
 GCG ACC CAC TCT CAA GGC TCG CCC ATT ACT TAT GCT A<sub>T</sub> A<sub>C</sub> GAC TAC GAT ACA ATG G<sub>T</sub> A<sub>G</sub> GAC CCC CTG GAG  
 A<sub>T</sub> Q<sub>S</sub> S<sub>E</sub> C<sub>E</sub> C<sub>S</sub>  
 4400\*  
 GCA GTG AGA CAG TCG GCT TIC G<sub>T</sub> A<sub>C</sub> GCT CAA ACC GGA GTG C<sub>T</sub> G ACG CTT AAT ATC CAG CCC ACG GCC A<sub>T</sub>  
 A<sub>A</sub> V<sub>R</sub> Q<sub>S</sub> L<sub>F</sub> K<sub>E</sub> F<sub>A</sub> G<sub>T</sub> V<sub>E</sub> A<sub>T</sub> G<sub>A</sub> G<sub>T</sub> A<sub>C</sub> GCT A<sub>T</sub> D<sub>A</sub> G<sub>T</sub> A<sub>C</sub> G<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub>  
 4500\*  
 ATG CAT GGA CTC TTC AAA TIC GAA GTC A<sub>T</sub> V<sub>E</sub> F<sub>A</sub> G<sub>T</sub> E<sub>A</sub> G<sub>T</sub> A<sub>C</sub> GCT GAC ACC GAC G<sub>T</sub> D<sub>A</sub> G<sub>T</sub> A<sub>C</sub> G<sub>T</sub> V<sub>E</sub> A<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub>  
 M<sub>H</sub> G<sub>G</sub> L<sub>F</sub> F<sub>A</sub> K<sub>E</sub> F<sub>A</sub> G<sub>T</sub> V<sub>E</sub> A<sub>T</sub> G<sub>A</sub> G<sub>T</sub> A<sub>C</sub> G<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub> C<sub>T</sub> G<sub>A</sub>  
 4600\*  
 TAC GTG GT<sub>A</sub> TCC TCG CAG AAC C<sub>G</sub> C<sub>G</sub> GTC TAC T<sub>T</sub> C GTC G<sub>T</sub> A<sub>A</sub> C<sub>G</sub> C<sub>G</sub> CAA C<sub>G</sub> G<sub>T</sub> C<sub>A</sub> C<sub>A</sub> C<sub>A</sub> A<sub>A</sub> G<sub>A</sub> G<sub>A</sub>  
 Y<sub>V</sub> S<sub>Q</sub> N<sub>R</sub> V<sub>F</sub> F<sub>A</sub> T<sub>C</sub> G<sub>T</sub> A<sub>C</sub> G<sub>C</sub> G<sub>T</sub> G<sub>A</sub> C<sub>A</sub> A<sub>A</sub> T<sub>G</sub> A<sub>C</sub> T<sub>G</sub> C<sub>A</sub> A<sub>A</sub> G<sub>A</sub> G<sub>A</sub> G<sub>A</sub>  
 4700\*  
 T<sub>T</sub> T<sub>F</sub> A<sub>T</sub> G<sub>A</sub> G<sub>C</sub>  
 GTC ACC GGC GTG GCG C<sub>T</sub> G GAG CAC A<sub>G</sub> C<sub>G</sub> C<sub>G</sub> A<sub>G</sub> C<sub>G</sub> A<sub>G</sub> C<sub>G</sub> A<sub>G</sub> C<sub>G</sub> T<sub>A</sub> C<sub>A</sub> T<sub>A</sub> G<sub>G</sub> A<sub>A</sub> C<sub>A</sub> T<sub>A</sub> C<sub>A</sub> T<sub>A</sub> G<sub>G</sub> C<sub>T</sub> G  
 V<sub>R</sub> G<sub>V</sub> A<sub>E</sub> H<sub>A</sub> S<sub>T</sub> Q<sub>M</sub> A<sub>A</sub> T<sub>A</sub> G<sub>G</sub> C<sub>T</sub> G  
 4800\*  
 ATG AGA TAGAACAGATCCGTAGCTGACCTAGCTCTCTGAGCTCCATACAAACGCTGGCTGGCGATCGGGGGTTGGAGGACCTGGTGGACCAAC  
 M<sub>R</sub>

**FIG. II**

4900 TCCAGCCCCGGAAGCTGTGACTCGAGGCCCTCACGGGTACGTCTGCCTCACTGTCTGGTTTCACTGTCTGGCTTGTGCTACTGCTTACCTTCATCAT  
5000 CAGGACTAGAGGGCTAAACCGACGGTTGGAAAGCCCTGTC6ATGACGAAGTGAGGGCTCACTGGACTCTGGATTGAACCGGGCATGCCGCCGGG  
5100 CACCAACAAACACACTGTGGAAAGGCTCCAAACCTATCTTCAATGAACCCAATAAAGACGCCAGATTAGATGCCATTAGC6AGGGTCCAACGACTCTGAT  
5200 CTGATCGGCATCGGAAGATCTTCCCACCTTGGCAACCACTTCTGAGGTGAACGAAAAGGCAAATCCTTATCCCGAAGTCGCAAACCAACAA<sup>9/12</sup>  
5300 CAACTTCGGC1TTCAACC6ACTCCCTCTGCCTGAGTTGTTAACGGACAGTTGAGAAAGATCTAGAAGATAACAAACACTAGTTAAGATCATTAA<sup>TTT</sup>  
5400 GGAGTTGGAAATTAAAGATTGTGATAAGAATGTGATTTTAAACTGTAATTCAAAAAAAATTGAGGACCTCCATTAAAGCTCTT  
5500 GCTCTCATCAAAATTATAAATGCCATTAGTCATTAAAGATAACTCGATTAACTTAAAGATTATGAAATTATATTGTC

FIG. II

MAVDVR1AAFLLVF1APAVLAQERCGYMT1PRLPRPDNL1PV1NFEQ1TWSORPL1PAPERDDLCMDAYH1T1ANLGTQV1YMD1EE1DE1IT1AI1NYNG 100  
 cad1  
 cad2  
 cad2  
 P1STPF1ELPFLSGSYNLLMPV1RRVDNGSASHHHARQHYELPGMQQYMFNVRDGS1VAGVSLA1VN1DDNAPI1QNFEP1CRVPELGE1PGL1TECTYQVS 200  
 cad3  
 cad3  
 DADGR1S1TEFMTFR1DSV1RGDEETFY1ERTN1PNQWMM1N1MT1GVNT1SLNFV1TSPLH1F1S1V1T1D1SLP1NT1HTV1TMM1VQVAN1VNSR1P1R1W1E1F1AV1Q1F1EE 300  
 cad4  
 cad4  
 KSYQNF1TVRA1DGD1T1NEED1FFS1E1ALP1GG1SGAVFL1SP1D1RT1Q1REV1F1PL1T1V1AY1K1D1E1A1F1S1T1N1V1V1I1V1D1N1Q1R1P1E1H1K 400  
 cad5  
 cad5  
 EYRLA1M1E1T1PL1N1F1D1K1E1F1G1H1D1K1D1L1G1Q1A1Q1Y1V1R1L1E1S1V1D1P1D1P1G1A1E1F1D1Y1E1V1P1E1F1O1S1T1R1W1V1G1V1A 500  
 LV1H1D1L1N1W1D1Q1P1F1E1H1A1V1Q1T1V1F1D1E1G1E1G1F1V1A1V1A1H1D1R1D1G1D1V1E1H1T1L1G1M1A1W1N1F1L1D1K1L1G1D1R1V1S1A1N1D1F1N1P1F1 600  
 cad6  
 cad6  
 HT1A1S1Q1L1V1R1N1D1N1N1T1P1P1T1L1P1R1G1S1P1Q1V1E1E1N1P1A1H1V1I1T1Q1E1R1F1E1I1D1S1A1T1K1G1R1G1A1N1P1D1F1R1M1C1Y1E1T1F1P1E1I1N1R1G1A1G1R1 700

**FIG. 2A-1**

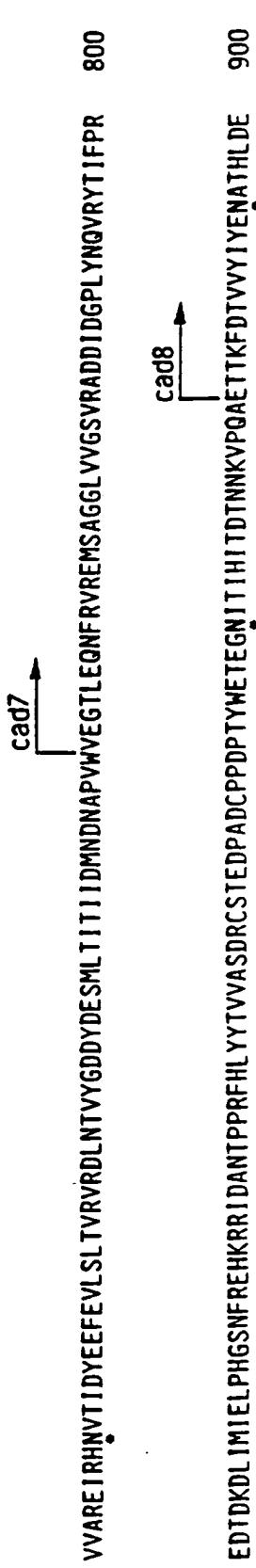
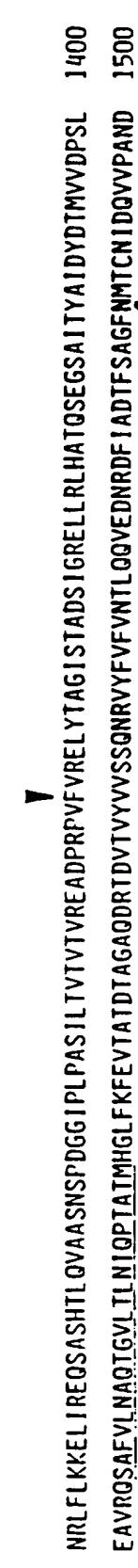
VVAREIRHNVVTIDYEEFEVLSLTVRVRDLNTVYGDYDESMLTITIDMNDNAPVWVEGTLQNFYREMSAGGLVVGSRADDIDGPLYNQVRYTIFPR 800  
  
 EDTDKDLIMIELPHGSNFREHKRRIDANTPPRFHLYYTVAASDRCSTEDPADCPPDPPTYMETEGNITIHTIDTNMKVQPAETTKFDTVYYIYENATHLDE 900  
 VVTLJASDLDREIYHMVSYVINYAVNPRLMNFFSVNRETGLYYDYETOGSGEVLDRDGDEPTHRIFNLIDNFMGEGEGRNRNQNDTEVLVILLDVNDN 1000  
 APELPPPSELSTISENLKGYVRLEPHIFAPDRDEPDTDNSRSGYEILNLSTERDIEVPELFVMIQIANVTGELETAMDLKGYWGTYAIYILAFDHGIPQ 1100  
 11/12  
 MSMMNETYELIHPFNYYAPEFFVFTINDAVIRLARERAVINGVLATVNNGEFLERISATDPDGLHAGVVTFOVVGDEESQRYFQVVVNDGENL GSLRLLUQAVP 1200  
  
 EEIREFRIFTIRATDQGTDPGPLSTDMDTFRVVVFVPTQGEPRFASSEHAVAFIEKSAGMEESHOLPLAODIKNHLCEDDCHSIYYRIIDGNSEGHFGLDPVR 1300  
  
 NRLFHKELIREQSAASHTLQVAASNNSPDDGIPLPASILTVTVTREADPRFVRELTYTAGISTADSIGRELLRLHATQSEGSAITYADYDTMVVDPSL 1400  
 EAVROSAEVLNAQITGYLTINIOPTATMHGLKFEVTAIDAGAQDRTDVTYVSSQNRVYFVFVNTLQQVEDNRDFIADTFSAGFNMTCNIDQWVVPAND 1500  
 PV1GVALEHSTOMAATSYGTTTYPYSLMR 1528

FIG. 2A-2

12/12

mp EC1	GPFPORNLNOL	RSINXFRGTTKIFYYSITGPAGADSPPEGVFTIEKES
fat EC18	GYQYQQIV	ARDADLQNAQLSYGVVSNDWANDVFSLNPNQT
pc42 EC2	GSLFPPIPL	ASDRDANELOQVAEDQEEKQPPQIVM
HPT-1 EC1	STPHPPIKIQ	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-1	IVTENIWKAPKPV	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-2	ITANLGTQVYMDEEJED	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-3	QNFEPICRVP	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-4	LEJFAVQOFE	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-5	IHKKEYRLAIM	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-6	EHAVQTVTFD	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-7	RLPRGSPQVE	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-8	VEGTLEQNFRR	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-9	ETTKFDTVVVY	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-10	PPPSELSWTIS	ASDRDANELOQVAEDQEEKQPPQIVM
BIRcad-11	VFPTNDAVIRLAR	ASDRDANELOQVAEDQEEKQPPQIVM
	ERAVIN	ASDRDANELOQVAEDQEEKQPPQIVM
	ASSEHAAVFI	ASDRDANELOQVAEDQEEKQPPQIVM

Cathelin Consensus Motif -----E-----6-----A.D.D.-----

**FIG. 2B-1**

mp EC1	SYVEEPMNISIIVT	SYVEEPMNISIIVT
fat EC18	LDREKIVKYELYGHAVSENGA	LDREKIVKYELYGHAVSENGA
pc42 EC2	LDYEVQGHYILLQAAQDNGQP	LDYEVQGHYILLQAAQDNGQP
HPT-1 EC1	LDREERWDSYDLIKVQDGGSP	LDREERWDSYDLIKVQDGGSP
BIRcad-1	LDREKDAYFYAVAKDEYQK	LDREKDAYFYAVAKDEYQK
BIRcad-2	LDREHYELPGM0QYMFNVRYD	LDREHYELPGM0QYMFNVRYD
BIRcad-3	LDRETFYIERTNIPNQWMNLNM	LDRETFYIERTNIPNQWMNLNM
BIRcad-4	LDYEVPEFQOSITIRVATDNNDT	LDYEVPEFQOSITIRVATDNNDT
BIRcad-5	LDYEVSEELFVQVRAIDLIGQP	LDYEVSEELFVQVRAIDLIGQP
BIRcad-6	LDYEEFEVSLTVRVDLNIVG	LDYEEFEVSLTVRVDLNIVG
BIRcad-7	LDYETGNTIHDNQNDTEVLVIL	LDYETGNTIHDNQNDTEVLVIL
BIRcad-8	LDYVVDYETQGSG	LDYVVDYETQGSG
BIRcad-9	LDYELLNLSSTERDIEPELFVUMIQIANVGELETAMDLKGWYGTYAIYI	LDYELLNLSSTERDIEPELFVUMIQIANVGELETAMDLKGWYGTYAIYI
BIRcad-10	LDYPLSTDMIFRUVFVPTOGEPF	LDYPLSTDMIFRUVFVPTOGEPF
BIRcad-11	LDYRDSASHTLQVAASNSPDGGI	LDYRDSASHTLQVAASNSPDGGI

Cathelin Consensus Motif  
6-----D.P.E.-----**FIG. 2B-2**Cathelin Consensus Motif  
6-----D.N.D.. P.F-----

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/13256

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.  
US CL :536/23.5; 435/7.21, 69.1, 240.2; 530/387.1; 514/2  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/7.21, 69.1, 240.2; 530/387.1; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, AGRICOLA, EMBASE, MEDLINE, DERWENT, CAS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 268, No. 17, issued 15 June 1993, VADLAMUDI et al, "A Specific Binding Protein from Manduca sexta for the Insecticidal Toxin of Bacillus thuringiensis subsp. berliner", pages 12334-12340, see the entire document.	1-14
X, P	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 270, No. 10, issued 10 March 1995, VADLAMUDI et al, "Cloning and Expression of a Receptor for an Insecticidal Toxin of Bacillus thuringiensis", pages 5490-5494, see the entire document.	1-14

Further documents are listed in the continuation of Box C.

See patent family annex.

•	Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"A"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search  
19 JANUARY 1996

Date of mailing of the international search report

13 FEB 1996

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer  
BRUCE CAMPBELL  
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/13256

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (6):

G01N 33/567; C12P 21/06; C12N 5/00; A01N 37/18; C07K 16/00; C07H 21/04